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Concentrations of Cd, Cu, Mn, Pb, and Zn in Fishes in a Highly Organic Softwater Pond

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Concentrations of Cd, Pb, and three essential metals (Cu, Mn, and Zn) in stocked bluegill (*Lepomis macrochirus*) and eight resident species of fish were studied in an acidic, highly organic pond on the southeastern U.S. coastal plain. Concentrations of Cu, Mn, and Zn in stocked and resident fish were apparently homeostatically controlled in liver tissue, axial musculature, and whole body. Concentrations of all metals studied in axial muscle tissue and whole body of stocked bluegill remained relatively constant after 200 d of residence in the pond. Analysis of concentration factors from pond water to whole stocked bluegill indicated that Pb in this system was less available to fish than in hardwater lakes. In contrast, Cd in the pond was as available to fish as in harder waters. Differences in relative availabilities of Cd and Pb were explained by the tendency of Pb to form complexes with naturally occurring organics. The need for metal-specific analysis of biological availability of metals in highly organic softwater systems is stressed.

Key words: trace metal, availability, fish, soft water, concentration factor, organic water

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Nous avons étudié les concentrations de Cd, Pb et trois métaux essentiels (Cu, Mn et Zn) dans des crapets arlequins introduits et huit espèces de poissons résidentes dans un étang acide, fortement organique, de la plaine côtière du sud-est des É.-U. Les concentrations de Cu, Mn et Zn dans les poissons introduits et résidents sont apparemment contrôlées homéostatiquement dans le tissu hépatique, la musculature axiale et le corps entier. Les concentrations de tous les métaux étudiés dans le tissu du muscle axial et dans le corps entier des crapets arlequins introduits sont demeurées relativement constantes après 200 jours de résidence dans l'étang. Une analyse des facteurs de concentration entre l'eau de l'étang et les crapets arlequins introduits entiers indique que le Pb dans ce réseau est moins accessible aux poissons que dans les lacs d'eau dure. Par contraste, le Cd dans l'étang est aussi accessible aux poissons que dans les eaux plus dures. Nous expliquons les différences dans la disponibilité relative du Cd et du Pb par la tendance du Pb à former des complexes avec des matières organiques présentes naturellement. Nous soulignons le besoin d'une analyse, métal par métal, de la disponibilité biologique des métaux dans des réseaux d'eau douce, fortement organique.

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AVAILABILITY and toxicity of trace metals to aquatic biota are primarily determined by the chemical nature of the aquatic environment. Most investigations of trace metal dynamics and bioavailability in freshwaters have been conducted in hardwater systems in which inorganic ligands, usually carbonate (Pagenkopf et al. 1974), control toxicity and availability of metals to biota. Freshwaters of the U.S. southeastern coastal plain are characterized by low alkalinity, hardness, and pH and high concentrations of dissolved humic and fulvic acids (Beck et al. 1974). Relatively few investigations o: trace metal dynamics have been conducted in acidic highly organic waters and few of these have attemptec to assess availability of trace metals to biota. Becaus naturally occurring organic acids can affect both toxicit and availability of trace metals to biota (Zitko et al 1973; Brown et al. 1974; Milanovich et al. 1975; Georg and Coombs 1977; Giesy et al. 1977), information o availability of metals in these systems is essential.

The primary objective of this study was to compar availability to fish of selected trace metals in an acidic highly organic pond (Skinface Pond) with availabilit in hardwater systems. Five metals, Cd, Cu, Mn, Pb, an Zn, were studied. Copper, Mn, and Zn are essentia trace metals and are presumably homeostatically con trolled (Bowen 1972; Frieden 1972; Cross et al. 1973 Bryan 1976; Giesy and Wiener 1977). Cadmium an Pb are nonessential, toxic metals (Davies et al. 1976

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Fleischer et al. 1974). Although essential, Cu and Zn can be acutely toxic at higher concentrations (Skidmore 1964; Black et al. 1976). Manganese is relatively nontoxic to aquatic biota and is seldom a problem in freshwaters (USEPA 1975). Analyses of these metals were conducted on eight species of resident fishes from the pond and on stocked bluegill (*Lepomis macrochirus*) that were collected at various time intervals following release into Skinface Pond. Comparison of availability was accomplished by analysis of *concentration factors*. However, concentration factors, defined as the ratio of trace metal concentration in the organism to that in water, give no indication of relative availability among various aquatic systems if the concentration of a trace metal in fish is regulated and independent of concentrations in water. Whole body concentrations of Cu, Mn, and Zn in resident fishes from Skinface Pond were therefore compared with values reported for the same species in other aquatic systems. Similarity of concentrations of a trace metal between differing aquatic systems was accepted as evidence of homeostatic regulation of concentrations of that metal by fish, and concentration factors were not used for assessing availability of that metal. Relationships between concentrations of these metals in whole body, axial musculature, and liver and body size of fishes were also examined for evidence of homeostatic regulation during growth (Cross et al. 1973).

Materials and Methods

Skinface Pond (33°14′N, 81°47′W) is located near Jackson, South Carolina, and adjacent to the U.S. Department of Energy's Savannah River Plant. The shallow (maximum depth ≈2.5 m), 2-ha pond receives no direct inputs of either industrial or domestic wastes. Water in the pond is darkly stained with organic acids during most of the year. The watershed draining into the pond has an estimated area of 14 km^2 and is managed by the U.S. Forest Service. Upland areas contain 25-yr-old stands of loblolly pine (*Pinus taeda*) and lowland areas contain 50-yr-old stands of mixed hardwoods. The watershed has very low relief, and soils are sandy, highly leached ultisols. Additional description of the regional area has been given by Langley and Marter (1973).

Resident fishes from Skinface Pond were collected on May 22, 1975 after drawdown of the pond and application of rotenone. Only fish that were collected immediately after surfacing were used for trace metal analysis. Fish were transported to the laboratory in ice-filled chests, placed in polyethylene bags, and stored at −4°C until lyophilization. The pond was treated with rotenone again on October 2, 1975 to remove fish that had invaded the pond since the first treatment. None of the fish from the second rotenone treatment was analyzed for trace metals. Replacement of pond water after drawdown and rotenone treatment had little effect on water quality because the pond had a high flushing rate and was not thermally stratified during late autumn and winter. On November 21, 1975, the pond was stocked with 3200 fingerling bluegill and redear sunfish (*L. microlophus*) obtained from the U.S. Fish and Wildlife Service National Fish Hatchery at Orangeburg, S.C. Samples of hatchery-reared, fingerling bluegill and redear sunfish were saved for trace metal analysis. Samples of stocked bluegill were collected at periodic intervals with seines and fish traps. These fish were transported live to the laboratory in polyethylene bags containing pond water and stored in polyethylene bags at −4°C until lyophilization. Despite intensive collection efforts, too few redear sunfish were obtained for analysis.

Water samples were collected monthly in a nonmetallic Kemmerer water sampler at surface, middepth, and 30 cm above bottom from three stations located at the deep end, center, and shallow back end of the pond. Water samples for trace metal analysis were acidified to 2% of volume with redistilled HNO_3 and stored at 4°C in washed polyethylene bottles until analyzed by flameless atomic absorption spectrophotometry, as described by Briese and Giesy (1975), Giesy and Briese (1977), and Giesy et al. (1978). Total organic carbon in pond water was determined with a Beckman model 915 carbon analyzer. Dissolved oxygen, temperature, specific conductance, redox potential, and pH were determined monthly at several depths at each station with a Hydrolab Surveyor model 6D water quality analyzer. Total alkalinity was determined in the field by titration. Binding capacity of surface water from Skinface Pond were determined for Ca, Cd, Cu, and Pb as described by Giesy et al. (1977).

Liver and axial muscle tissues of fish were dissected with stainless steel implements on a clean polyethylene work surface. Polyethylene gloves were worn during dissection of fish tissues to reduce surface contamination of samples. After dissection, tissue samples were placed in washed and tared plastic vials, weighed, and lyophilized to a constant dry weight. Whole fish were dried, digested, and analyzed by atomic absorption spectrophotometry as described by Giesy and Wiener (1977). Methods for cleaning glassware, porcelain crucibles and polyethylene bottles used for sample digestions and storage have also been described (Giesy and Wiener 1977). Both standard additions and additions of NH_4NO_3 were made during Pb determinations to allow partial elimination of matrix interferences by increasing charring (pyrolysis) temperature (Briese and Giesy 1975). Sample preparation and analytical procedures were evaluated and validated, using U.S. National Bureau of Standards (NBS) bovine liver as a reference material. Our results were within the range of concentrations given by NBS for each metal studied.

Blanks used to assess contamination from reagents and container walls do not allow correction for surface contamination resulting from handling of samples (e.g. dissection). Therefore, an additional, statistical approach was employed for each species, metal, and sample type (whole fish, muscle, and liver) to evaluate sample contamination during handling. For each species and sample type, digested samples were diluted to known volumes. For a given species and sample type, the concentrations (corrected for analytical blanks) of each metal in these solutions should be approximately proportional to the respective biomass:dilution volume ratios, barring surface contamination of the samples. For samples diluted to equal volumes, the corrected concentrations of trace metals in the solution should be proportional to the biomass of sample digested. Hence, for each species and sample type, a simple linear regression of blank-corrected metal concentration against sample biomass (or biomass:dilution volume ratio) should yield a regression equation with a significant, positive slope and an intercept that does not differ significantly from zero. Although concentrations of a given trace metal can be highly variable within a given species and sample type, a positive correlation between solution concentration and sample biomass (or biomass:dilution volume ratio) should be demonstrable across a wide range of sample biomass. Given adequate sample size, lack of a positive regression slope, or occurrence of a significant, nonzero intercept suggests surface contamination during sample handling.

Data analyses were conducted with the Statistical Analysis System (Barr et al. 1976). A Type I error of 5% was used to judge significance of statistical tests. In cases of significant analysis of variance, multiple comparison of means were made with Scheffe's test.

Results and Discussion

Analysis of Pond Water

Skinface Pond underwent marked vertical stratification during spring and summer, and the hypolimnion was anoxic during most of this time. Therefore, only data on water from strata containing sufficient dissolved oxygen for fish life were included in the statistical analysis (for inclusion, dissolved oxygen $\geq$3.98 mg/L, the mean threshold of incipient oxygen response reported for nonsalmonid freshwater fishes by Davis (1975)). Hence, means in Table 1 are estimates of annual averages of the measured parameters in the portion of the water column inhabited by fish. Results show that Skinface Pond is typical of freshwaters of the southeastern coastal plain (Beck et al. 1974). The pond is poorly buffered, acidic, and has very low concentrations of dissolved ions (Table 1). Total organic carbon measured in oxygenated surface water ranged from ~15 mg/L to >30 mg/L, indicating the highly organic nature of the pond. Additional chemical description of surface water from Skinface Pond has been given by Giesy et al. (1977).

TABLE 1. Water chemistry data from Skinface Pond. Statistics for all parameters except binding capacity were calculated from average monthly determinations at surface, middepth, and bottom at three stations on the pond over a 1-yr period. Only data from strata containing sufficient dissolved oxygen for fish life were included in the calculations.

Parameter	Mean	Range	C.V. (%)
Temperature (°C)	19	5–31	50
Dissolved oxygen (mg/L)	6.4	4.6–9.2	20
Total alkalinity (mg $CaCO_3$/L)	2.1	1.4–3.0	29
Specific conductance (micromhos/cm)	39	27–58	30
Redox potential (mv)	460	375–525	12
pH	4.6	4.2–5.0	6
Metal concentrations (μg/L)[a]			
Cd	0.17	0.02–0.41	60
Cu	7.6	1.0–15.4	70
Mn	91	39–151	37
Pb	14.1	1.3–34.2	69
Binding capacity (μg-atoms/L)[b]			
Ca	14.3	—	—
Cd	5.2	—	—
Cu	15.0	—	—
Pb	21.0	—	—

[a]Unfiltered water samples.
[b]Pond water with particulates (>0.15 μm) removed.

Evaluation of Contamination of Fish Samples

Blanks used to assess contamination from reagents and container walls indicated substantial sample contamination with Cd but not with Cu, Mn, Pb, or Zn. This applied to all sample collections of resident fishes (from rotenone treatment) and of two sample collections of whole, stocked bluegill. Cadmium concentrations are therefore not reported for these samples. Later investigation showed that Cd contamination occurred during digestion of samples through leaching of Cd from pyrex watch glasses under conditions of high temperature and strong acid.

Linear regression of blank-corrected solution concentration against sample biomass or sample biomass:dilution volume ratio indicated surface contamination of liver and axial muscle samples, though not of whole fish, with Pb. The regression technique indicated no contamination of whole fish or fish tissues by Cu, Mn, or Zn and no surface contamination of whole fish or muscle tissue by Cd in those samples not contaminated with Cd during digestion. Typical regressions are shown for resident warmouth (*L. gulosus*) in Fig. 1. Surface contamination of axial muscle and liver tissues by Pb probably occurred during dissection, due to contact of samples with mucosal surface slime, which contains high concentrations of Pb relative to muscle

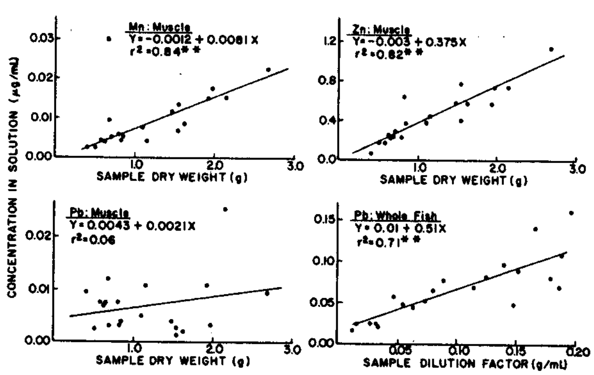

FIG. 1. Checks for surface contamination of fish samples, exemplified for resident warmouth (*Lepomis gulosus*) from Skinface Pond. Surface contamination by Pb is indicated for muscle but not for whole fish samples, whereas no contamination of muscle samples by Mn or Zn is evident (see text). Two asterisks (**) indicate a regression slope that differs from zero at the 1% level of significance. None of the intercepts differ significantly from zero.

TABLE 2. Mean whole body concentrations of Cu, Mn, Pb, and Zn in seven species of resident fishes from Skinface Pond. Concentration ranges are given in parentheses under each mean. Means for a given element that do not contain the same letter in their superscripts were judged significantly different ($\alpha = 0.05$). *Values can be changed to wet weight concentrations by multiplying by 0.25, the mean dry wt:wet wt ratio for whole fish.

Species	n	Dry wt range of fish (g)	Concn (μg/g dry wt)* Cu	Mn	Pb	Zn
Bluegill	17	0.11–82.31	2.15[a] (1.14–5.91)	40.4[ab] (14.7–90.7)	1.1[a] (0.3–4.3)	142[bc] (56–582)
Warmouth	21	0.62–75.98	2.04[a] (1.04–3.70)	12.8[b] (7.5–21.3)	0.8[abc] (0.3–1.6)	103[cd] (71–183)
Largemouth bass	22	1.34–277.45	1.88[a] (1.06–5.30)	4.5[b] (2.3–19.0)	0.5[bc] (0.1–1.7)	75[d] (42–181)
Chain pickerel	20	0.43–150.26	1.60[a] (1.14–4.19)	14.6[b] (6.6–58.3)	0.3[c] (0.05–1.4)	209[ab] (138–310)
Redfin pickerel	16	0.35–26.04	2.77[a] (1.18–6.57)	19.3[b] (5.4–59.0)	1.1[ab] (0.2–3.0)	216[a] (126–296)
American eel	13	0.98–88.21	2.34[a] (1.30–6.43)	9.7[b] (5.0–16.0)	0.4[bc] (0.2–1.2)	75[cd] (61–89)
Lake chubsucker	18	16.83–113.79	2.64[a] (1.67–3.80)	75.1[a] (24.0–342.0)	0.4[c] (0.2–0.6)	79[cd] (54–102)

and liver (Patterson and Settle 1977). Concentrations of Pb in axial muscle and liver tissue are consequently not presented. A detailed description of precautions for reducing sample contamination by Pb during collection, handling, and analysis has since been published by Patterson and Settle (1976).

TRACE METAL CONCENTRATIONS IN RESIDENT FISHES

Whole body concentrations of Cu, Mn, Pb, and Zn were determined for samples of seven of the more abundant species of fish from the pond, bluegill, warmouth, largemouth bass (*Micropterus salmoides*), chain pickerel (*Esox niger*), redfin pickerel (*Esox americanus americanus*), American eel (*Anguilla rostrata*), and lake chubsucker (*Erimyzon sucetta*) (Table 2). A one-way analysis of variance (ANOVA) indicated significant species effects on whole body concentrations of all four metals. Results of analysis of covariance, with total length as a covariate, were similar. Total length was a significant covariate for Cu, Pb, and Zn and species effects were highly significant ($P < 0.01$) for all four metals after adjusting for length. Copper exhibited less variation among species means than the other metals. Although ANOVA indicated significant species effects on the whole body concentrations of Cu, no two species' means differed significantly, due to the conservative nature of Scheffe's test (Kirk 1968, p. 95).

Mean whole body concentrations of Cu, Mn, and Zn in fish from Skinface Pond were generally similar to the limited data reported elsewhere for samples of the same species collected from relatively uncontaminated systems, suggesting homeostatic regulation of these metals in the whole body. Mean concentrations of Mn and Zn in largemouth bass from five diverse freshwater systems in the southeastern U.S. (Goodyear and Boyd 1972) are approximately equal to mean concentrations given here for that species. However, the mean Cu concentration reported by Goodyear and Boyd (1972) for the five localities (grand mean = 6.2 μg/g) is much higher than that reported here. The mean Cu (2.19 μg/g) and Zn (173 μg/g) concentrations in bluegill from nearby Par Pond (Giesy and Wiener 1977) are very close to those given here for bluegill from Skinface Pond. Mean whole body concentrations of Zn in bluegill (139 μg/g), warmouth (127 μg/g), and largemouth bass (79 μg/g) collected from the uncontaminated portion of a hardwater lake in Indiana (Murphy et al. 1978) were also similar to values given here for those species.

Mean Cu, Mn, and Zn concentrations were lower in axial muscle tissue than in liver tissue in bluegill, largemouth bass, chain pickerel, and bowfin (*Amia calva*) (Table 3). A one-way ANOVA indicated highly significant species effects on concentrations of all three metals for both liver and muscle tissue. Differences in mean concentrations among species were much less pronounced in muscle tissue than in liver tissue. Concentrations in axial muscle tissue were not correlated with concentrations in liver tissue for Cu, Mn, or Zn in either largemouth bass or chain pickerel. A positive correlation should have occurred if these metals were accumulating in the two tissues. Similarly, Pentreath (1976) found no correlation of concentrations of Fe, Mn, or Zn among muscle, liver, and bone tissues of European plaice (*Pleuronectes platessa*).

No species exhibited consistently higher or lower

TABLE 3. Mean concentrations of Cu, Mn, and Zn in axial muscle and liver tissue of resident fishes from Skinface Pond. Concentration ranges are provided in parentheses under each mean. Means for a given element and tissue that do not contain the same letter in their superscripts were judged significantly different ($\alpha = 0.05$). *Values can be changed to wet weight concentrations by multiplying by 0.20 and 0.24, respectively, for axial muscle and liver tissue.

Sample/species	n	Live wt range of fish (g)	Concn (μg/g dry wt)* Cu	Mn	Zn
Axial muscle					
Bluegill	12	97.7–351.2	0.62^{ab} (0.35–1.12)	0.42^{b} (0.23–0.70)	31.7^{a} (12.3–48.7)
Warmouth	20	15.7–236.4	0.61^{b} (0.26–1.99)	0.45^{b} (0.18–2.33)	18.4^{b} (9.0–37.3)
Largemouth bass	19	51.3–1191.8	1.08^{a} (0.46–2.52)	0.32^{b} (0.13–0.59)	12.7^{b} (7.0–24.5)
Chain pickerel	16	94.9–773.7	0.29^{b} (0.06–0.88)	1.21^{a} (0.19–2.30)	14.7^{b} (4.9–32.8)
Bowfin	4	1192–2561	0.85^{ab} (0.49–1.48)	0.87^{ab} (0.65–1.40)	22.2^{ab} (18.0–26.3)
Liver					
Bluegill	4	192.5–285.8	4.8^{b} (4.1–5.6)	7.18^{a} (2.58–12.96)	39.7^{b} (21.3–55.4)
Largemouth bass	17	178.5–1191.8	24.8^{b} (6.7–105.8)	3.69^{b} (2.24–5.55)	74.4^{b} (18.9–173.9)
Chain pickerel	13	94.9–396.5	27.8^{b} (10.2–54.4)	1.77^{c} (0.80–2.68)	337.9^{a} (150.0–855.6)
Bowfin	4	1192–2561	548.1^{a} (250.8–918.9)	4.97^{ab} (2.99–7.05)	85.7^{b} (45.7–121.3)

concentrations of Cu, Mn, and Zn in either whole body, axial musculature, or liver tissue, and no trophic level biomagnification of Cu, Mn, Pb, and Zn was observed among fishes of different trophic position. Species differences in trace metal concentrations of fish tissues and/or whole fish from a single locality have been reported elsewhere (Lucas et al. 1970; Cross and Brooks 1973; Lentsch et al. 1973; Ting 1973; Gilmartin and Revelante 1975; Hutchinson et al. 1976; Giesy and Wiener 1977; Murphy et al. 1978). Except for Hg (Leland et al. 1977), there has been essentially no indication of higher concentrations of trace metals in fishes of higher trophic status relative to fishes of lower trophic status (Mathis and Cummings 1973; Ting 1973; Stevens and Brown 1974; Hutchinson et al. 1976; Giesy and Wiener 1977; Murphy et al. 1978). In addition, fishes having similar food habits may exhibit marked differences in whole body (Cross and Brooks 1973) or tissue concentrations (Ting 1973) of certain trace metals. Adult chain pickerel and largemouth bass are primarily piscivorous; however, mean trace metal concentrations for these species in Skinface Pond often differed significantly (Tables 2 and 3). Trace metal concentrations in fish are apparently related to factors other than food habits and trophic status.

Results of correlational analyses between body size and Cu, Mn, and Zn concentrations provided further empirical evidence of homeostatic regulation of these metals in fish. We found negative correlations between whole body concentrations of Cu, Mn, and Zn and total length for all species analyzed except lake chubsucker (Table 4). Decreases in whole body concentrations of essential trace metals with increasing body size of fish have been reported elsewhere (Cross and Brooks 1973; Bohn and McElroy 1976; Giesy and Wiener 1977; Murphy et al. 1978). However, increasing whole body concentrations of Zn with body size of fish have also been observed (Bohn and McElroy 1976; Cutshall et al. 1977). Cross and Brooks (1973) suggested that decreases in whole body concentrations with size result from shifts in the relative proportions of body tissues that have differing metal concentrations.

Copper, Mn, and Zn concentrations in liver and axial muscle tissue were generally not correlated with body size of fish from Skinface Pond (Table 4), similar to results reported elsewhere (Cross et al. 1973; Eustace 1974; Mathis and Kevern 1975; Pentreath 1976; Bohn and McElroy 1976).

TRACE METAL CONCENTRATIONS IN STOCKED BLUEGILL

A one-way ANOVA indicated highly significant temporal changes in whole body concentrations of Cu, Pb, and Zn in bluegill that were stocked into Skinface Pond, but no trend of increasing concentrations with time was apparent (Table 5). Mean whole body con-

TABLE 4. Correlation coefficients (r) between total length and dry weight concentrations of trace metals in whole body, axial muscle tissue, and liver tissue of resident fishes from Skinface Pond. Only significant correlation coefficients ($\alpha = 0.05$) are given. Nonsignificant correlations are indicated by ns; *r significant at the 5% level; **r significant at the 1% level.

Sample and species analyzed	n	Cu	Mn	Pb	Zn
Whole body					
Bluegill	17	−0.68**	ns	−0.48*	−0.79**
Warmouth	21	−0.68**	ns	−0.66**	−0.84**
Largemouth bass	22	−0.72**	−0.63**	−0.53*	−0.84**
Chain pickerel	20	−0.70**	−0.61**	−0.56*	ns
Redfin pickerel	16	ns	−0.60*	ns	−0.79**
American eel	13	−0.89**	ns	−0.91**	ns
Lake chubsucker	18	ns	ns	ns	ns
Muscle					
Bluegill	12	ns	ns	—	ns
Warmouth	20	ns	ns	—	ns
Largemouth bass	19	ns	−0.48*	—	ns
Chain pickerel	16	ns	ns	—	ns
Liver					
Largemouth bass	17	ns	ns	—	0.60*
Chain pickerel	13	ns	ns	—	ns

centrations of Zn in bluegill decreased and remained constant at approximately 100 μg/g dry wt as of 220 d after stocking. Whole body concentrations of Cu and Pb were highest in fingerling bluegill collected 97 d after stocking and decreased to relatively constant levels in samples collected during and after 220 d. Cadmium and Mn concentrations were relatively constant in whole bluegill throughout the collection period.

One-way ANOVA indicated highly significant temporal variation in Mn and Zn concentrations in axial muscle tissue of bluegill collected 216, 360, and 511 d after stocking (Table 6). Residence time of stocked bluegill in the pond did not significantly affect Cd or Cu concentrations in axial muscle tissue. Hence, there was no general trend of increasing concentrations of the four metals in muscle tissue with time.

Whole body Cu and Zn concentrations were higher ($P < 0.01$, t-test) whereas Mn and Pb concentrations were lower ($P < 0.05$ for Pb, $P < 0.01$ for Mn) in stocked bluegill recaptured after 499 d than in larger (dry wt > 5 g, $n = 12$) resident bluegill collected during rotenone treatment. Only mean whole body concentrations of Mn differed between resident and stocked bluegill by a factor of more than 2. In contrast to whole body concentrations, mean Cu, Mn, and Zn concentrations in muscle tissue of resident (Table 3) and stocked bluegill (Table 6, time = 511 d) were similar and differed significantly only for Mn (t-test, $P < 0.01$). How-

TABLE 5. Mean whole body concentrations of trace metals in bluegill collected during and after stocking of Skinface Pond. Concentration ranges are provided in parentheses under each mean. Means for Cu, Pb, or Zn that do not contain the same letter in their superscripts were judged significantly different ($\alpha = 0.05$).

Time (days after stocking)	n	Dry wt range (g)	Concn (μg/g dry wt) Cd	Cu	Mn	Pb	Zn
0	12	0.02–0.41	0.07 (0.02–0.23)	11.5^{b} (2.8–63.3)	21.4 (14.4–44.8)	0.19^{b} (0.00–0.76)	349^{a} (177–660)
97	12	0.04–0.27	0.07 (0.04–0.12)	324.9^{a} (247.6–404.0)	20.7 (15.1–29.8)	1.22^{a} (0.43–5.49)	249^{b} (153–365)
220	15	3.64–13.97	—	2.5^{b} (0.9–4.4)	16.0 (8.9–18.9)	0.29^{b} (0.04–0.63)	106^{c} (95–124)
340	11	3.97–15.13	—	1.7^{b} (1.2–2.1)	18.2 (10.0–26.5)	0.14^{b} (0.04–0.30)	100^{c} (84–120)
499	10	6.6–23.8	0.04 (0.01–0.09)	2.8^{b} (1.6–3.9)	19.9 (14.2–25.7)	0.47^{ab} (0.12–0.76)	102^{c} (85–117)

TABLE 6. Mean dry weight concentrations of trace metals in muscle tissue of bluegill collected after stocking of Skinface Pond. Concentration ranges are provided in parentheses under each mean. Means for Mn or Zn that do not contain the same letter in their superscripts were judged significantly different ($\alpha = 0.05$).

Time (days after stocking)	n	Live wt range of fish (g)	Concn (μg/g dry wt)			
			Cd	Cu	Mn	Zn
216	13	29.6–57.1	0.0092 (0.0029–0.0361)	1.28 (0.46–5.78)	0.38[a] (0.18–0.58)	41.4[a] (31.5–57.4)
360	11	43.6–82.2	0.0040 (0.0008–0.0108)	0.52 (0.40–0.69)	0.39[a] (0.26–0.59)	34.8[b] (28.1–42.6)
511	11	61.3–124.9	0.0088 (0.0037–0.0164)	0.51 (0.38–0.66)	0.75[b] (0.60–0.95)	31.3[b] (24.6–38.2)

ever, the mean concentration of Mn in muscle tissue of resident bluegill was similar to those in stocked bluegill collected 216 and 360 d after release. Although whole body concentrations of Cu, Mn, and Zn differed between resident and stocked bluegill, average concentrations of these metals in muscle tissue from the two populations were similar. Concentrations of these trace metals in muscle tissue were apparently regulated. Concentrations of Cd, Cu, Mn, Pb (calculated without correcting for surface contamination), and Zn in muscle tissue were low, indicating that only small quantities of these metals were accumulated there.

Availability of Cd and Pb in Skinface Pond to stocked bluegill was compared with availability in hardwater systems with concentration factors (Jinks and Eisenbud 1972). Concentration factors (CF) between water and stocked bluegill were calculated with the formula

$$CF = \frac{C_o}{C_w}$$

where C_o = dry weight concentration of a given trace metal in the organism (bluegill), and

C_w = average total concentration of the trace metal in water. Annual average concentrations of metals in water (Table 1) were used for C_w for Skinface Pond, as suggested by Lentsch et al. (1973). In addition, C_w were determined from unfiltered water samples, as suggested by Vanderploeg et al. (1975). *CF* were determined for whole bluegill collected approximately 500 d after stocking (Table 7). These CF may be considered equilibrium values because metal concentrations in whole bluegill were relatively stable by approximately 200 d after stocking (Table 5). Because concentrations of trace metals other than Hg are strongly regulated in axial muscle tissue of fish (Phillips 1977), *CF* were not calculated for muscle tissue. *CF* for Cu and Mn in Table 7 were not used for comparative purposes because of homeostatic regulation. For comparison, *CF* for Cd and Pb were calculated for whole bluegill from three hardwater systems (total alkalinity >100 mg/L $CaCO_3$) in Indiana (Palestine Lake: ditch; Palestine Lake: east basin; and Little Center Lake) with data published by Atchison et al. (1977). Mean whole body concentrations for bluegill and estimated mean total concentrations in water (total = mean dissolved concentration + mean suspended concentration) were used in the calculations.

The slight differences in body size distributions between the samples of bluegill from Indiana Lakes and Skinface Pond should not affect our comparison of availability. Whole body concentrations of Cd were not correlated with body size in bluegill from either Palestine Lake (Murphy et al. 1978) or Skinface Pond (Table 5). Differences in the size distributions between samples of bluegill from Little Center Lake and Skinface Pond were small. Total length median and range for the sample from Little Center Lake were 115 and 81–177 mm, respectively, whereas median and range for the Skinface Pond sample were 154 and 118–163 mm, respectively. In addition, the relationship between Pb concentration and total length accounted for only 23% of the variation in Pb concentration in whole, resident bluegill from Skinface Pond (Table 4).

CF for Cd for the three Indiana sites were lower than the *CF* for whole bluegill from Skinface Pond (Table 7). In contrast, the *CF* for Pb in bluegill from Little Center Lake was much higher than for bluegill from Skinface Pond. Lead concentrations in water and bluegill from the other two sites in Indiana were below the detection limits of the methods employed by Atchison et al. (1977).

TABLE 7. Concentration factors (*CF*) for whole bluegill collected from Skinface Pond approximately 500 d after stocking, relative to *CF* for bluegill collected from hardwater lakes in Indiana by Atchison et al. (1977) (see text).

Element	*CF*	
	Skinface Pond	Hardwater Lakes
Cd	240	71[a], 83[b], 120[c]
Cu	370	—
Mn	220	—
Pb	33	230[c]

[a]Palestine Lake: ditch.
[b]Palestine Lake: east basin.
[c]Little Center Lake.

We assume that a greater *CF* for a metal indicates greater availability of that metal in the environment. The *CF* for Cd in Skinface Pond is approximately 2.6 times greater than the mean *CF* for Cd in the hardwater systems studied by Atchison et al. (1977). Although the statistical significance of this difference cannot be assessed, we believe that Cd in Skinface Pond is possibly more available, but at least as available to fish as in hardwater lakes. Proportionately more Cd may exist as the free divalent cation in acidic, highly organic waters than in hardwater systems because controls on Cd^{2+} concentration in surface waters are primarily inorganic, and very little Cd is bound to either organic or inorganic ligands in waters having low pH and high organic carbon concentration (Giesy et al. 1978). This may explain the greater availability of Cd in Skinface Pond, relative to hardwater lakes. In contrast, Pb in Skinface Pond was relatively unavailable to fish. A lower *CF* for Pb in a highly organic system is not surprising. The binding capacity of Skinface Pond water for Pb was about 4 times greater than that for Cd (Table 1). Most of the Pb in these waters is associated with high molecular weight organic acids and particulate matter (Giesy and Briese 1977) and is not in the ionic form (Pb^{2+}), which is readily available for direct uptake through the gills of fish (Holcombe et al. 1976; Merlini and Pozzi 1977a, b). Although it is unclear whether uptake from water or food accounts for the majority of Pb accumulation in fish inhabiting natural systems, Pb is less available to fish in Skinface Pond than in hardwater lakes. Because fish readily accumulate available (ionic) Pb (Holcombe et al. 1976; Merlini and Pozzi 1977a, b), we believe the negative correlations between whole body concentrations of Pb and body size in resident fishes (Table 4) further indicate low availability of Pb in Skinface Pond to fish.

In a recent review, Jenne and Luoma (1977) suggested that biotic accumulation of trace elements present at similar total concentrations would vary inversely with concentrations of dissolved organics. Although our findings for Pb support their suggestion, our findings for Cd indicate that it should not be generally applied to all trace metals and all waters. We stress the need for metal-specific analysis of biological availability of metals in highly organic, softwater systems.

Much of the information presented here indicates homeostatic regulation of Cu, Mn, and Zn in resident and stocked fish from Skinface Pond. Despite considerable differences in ambient metal concentrations and other chemical characteristics, mean whole body concentrations of Cu, Mn, and Zn in resident fishes from Skinface Pond were generally similar to values reported elsewhere for the same species and remained constant or decreased slightly with increasing body size. Also, Cu, Mn, and Zn concentrations did not vary with body size in liver tissue or axial musculature. Similarly, mean axial muscle and whole body concentrations of Cu, Mn, and Zn in stocked bluegill were relatively constant approximately 200 d after stocking and did not change substantially with growth. Hence, these metals did not accumulate in the tissues with age. Further evidence of homeostatic regulation of Cu, Mn, and Zn has been presented by Schroeder et al. (1966), Goodyear and Boyd (1972), Cross et al. (1973), Lentsch et al. (1973), Merlini et al. (1973), and Giesy and Wiener (1977).

These results have important implications for biological monitoring programs. An organism or group of organisms is of no value for biological monitoring of a trace metal if concentration of that metal in the organism is regulated and independent of ambient concentrations. Use of fish for such monitoring should therefore be limited to nonessential metals that accumulate in the body with exposure.

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ATCHISON, G. J., B. R. MURPHY, W. E. BISHOP, A. W. MCINTOSH, AND R. A. MAYES. 1977. Trace metal contamination of bluegill (*Lepomis macrochirus*) from two Indiana lakes. Trans. Am. Fish. Soc. 106: 637–640.

BARR, A. J., J. H. GOODNIGHT, J. P. SALL, AND J. T. HELWIG. 1976. A user's guide to SAS76. SAS Inst., Inc., Raleigh, N.C. 329 p.

BECK, K. C., J. H. REUTER, AND E. M. PERDUE. 1974. Organic and inorganic geochemistry of some coastal plain rivers of the southeastern United States. Geochim. Cosmochim. Acta 38: 341–364.

BLACK, G. A. P., D. J. HINTON, H. C. JOHNSTON, AND J. B. SPRAGUE. 1976. Annotated list of copper concentrations found harmful to aquatic organisms. Fish. Mar. Serv. Tech. Rep. 603: 44 p.

BOHN, A., AND R. O. MCELROY. 1976. Trace metals (As, Cd, Cu, Fe, and Zn) in Arctic cod, *Boreogadus saida*, and selected zooplankton from Strathcona Sound, northern Baffin Island. J. Fish. Res. Board Can. 33: 2836–2840.

BOWEN, H. J. M. 1972. The biochemistry of the trace elements, p. 393–405. *In* Nuclear activation techniques in the life sciences. Int. Atomic Energy Agency, Vienna.

BRIESE, L. A., AND J. P. GIESY JR. 1975. Determination of lead and cadmium associated with naturally occurring organics extracted from surface waters, using flameless atomic absorption. At. Absorpt. Newsl. 14: 133–136.

BROWN, V. M., T. L. SHAW, AND D. G. SHURBEN. 1974. Aspects of water quality and the toxicity of copper to rainbow trout. Water Res. 8: 797–803.

BRYAN, G. W. 1976. Some aspects of heavy metal tolerance in aquatic organisms, p. 7–34. *In* A. P. M. Lockwood [ed.] Effects of pollutants on aquatic organisms. Cambridge Univ. Press, London.

CROSS, F. A., AND J. H. BROOKS. 1973. Concentrations of manganese, iron, and zinc in juveniles of five estuarine-dependent fishes, p. 769–775. *In* D. J. Nelson [ed.] Radionuclides in ecosystems, Vol. 2, Proc. Third Nat. Symp. Radioecol., U.S. Atomic Energy Comm., Symp. Ser. CONF-710501.

CROSS, F. A., L. H. HARDY, N. Y. JONES, AND R. T. BARBER. 1973. Relation between total body weight and concentrations of manganese, iron, copper, zinc, and mercury in white muscle of bluefish (*Pomatomus saltatrix*) and a bathyl-demersal fish *Antimora rostrata*. J. Fish. Res. Board Can. 30: 1287–1291.

CUTSHALL, N. H., J. R. NAIDU, AND W. G. PEARCY. 1977. Zinc and cadmium in the Pacific hake *Merluccius productus* off the western U.S. coast. Mar. Biol. 44: 195–201.

DAVIES, P. H., J. P. GOETTL JR., J. R. SINLEY, AND N. F. SMITH. 1976. Acute and chronic toxicity of lead to rainbow trout *Salmo gairdneri*, in hard and soft water. Water Res. 10: 199–206.

DAVIS, J. C. 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. J. Fish. Res. Board Can. 32: 2295–2332.

EUSTACE, I. J. 1974. Zinc, cadmium, copper and manganese in species of finfish and shellfish caught in the Derwent Estuary, Tasmania. Aust. J. Mar. Freshwat. Res. 25: 209–220.

FLEISCHER, M., A. F. SAROFIM, D. W. FASSETT, P. HAMMOND, H. T. SHACKLETTE, I. C. T. NISBET, AND S. EPSTEIN. 1974. Environmental impact of cadmium: A review by the panel on hazardous trace substances. Environ. Health Perspect. p. 253–323.

FRIEDEN, E. 1972. The chemical elements of life. Sci. Am. 227: 52–60.

GEORGE, S. G., AND T. L. COOMBS. 1977. The effects of chelating agents on the uptake and accumulation of cadmium by *Mytilus edulis*. Mar. Biol. 39: 261–268.

GIESY, J. P. JR., AND L. A. BRIESE. 1977. Metals associated with organic carbon extracted from Okefenokee Swamp water. Chem. Geol. 20: 109–120.

GIESY, J. P. JR., L. A. BRIESE, AND G. J. LEVERSEE. 1978. Metal binding capacity of selected Maine surface waters. Environ. Geol. 2: 257–268.

GIESY, J. P. JR., G. J. LEVERSEE, AND D. R. WILLIAMS. 1977. Effects of naturally occurring aquatic organic fractions on cadmium toxicity to *Simocephalus serrulatus* (Daphnidae) and *Gambusia affinis* (Poeciliidae). Water Res. 11: 1013–1020.

GIESY, J. P. JR., AND J. G. WIENER. 1977. Frequency distributions of trace metal concentrations in five freshwater fishes. Trans. Am. Fish. Soc. 106: 393–403.

GILMARTIN, M., AND N. REVELANTE. 1975. The concentration of mercury, copper, nickel, silver, cadmium, and lead in the northern Adriatic anchovy, *Engraulis encrasicholus*, and sardine, *Sardina pilchardus*. Fish. Bull. 73: 193–201.

GOODYEAR, C. P., AND C. E. BOYD. 1972. Elemental composition of largemouth bass (*Micropterus salmoides*). Trans. Am. Fish. Soc. 101: 545–547.

HOLCOMBE, G. W., D. A. BENOIT, E. N. LEONARD, AND J. M. MCKIM. 1976. Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 33: 1731–1741.

HUTCHINSON, T. C., A. FEDORENKO, J. FITCHKO, A. KUJA, J. VAN LOON, AND J. LICHWA. 1976. Movement and compartmentalization of nickel and copper in an aquatic ecosystem, p. 565–585. *In* J. O. Nriagu [ed.] Environmental biogeochemistry, Vol. 2, Metals transfer and ecological mass balances. Ann Arbor Science Publishers Inc., Ann Arbor, Mich.

JENNE, E. A., AND S. N. LUOMA. 1977. Forms of trace elements in soils, sediments, and associated waters: An overview of their determination and biological availability, p. 110–143. *In* Biological implications of metals in the environment, U.S. Energy Res. Development Admin. Symp. Ser. CONF-750929.

JINKS, S. M., AND M. EISENBUD. 1972. Concentration factors in the aquatic environment. Radiation Data Rep. 13: 243–247.

KIRK, R. E. 1968. Experimental design: procedures for the behavioral sciences. Wadsworth Publishing Co., Belmont, Calif. 577 p.

LANGLEY, T. M., AND W. L. MARTER. 1973. The Savannah River Plant site. U.S. Atomic Energy Comm. Rep. DP-1323, E. I. du Pont de Nemours and Co., Savannah River Lab., Aiken, S.C. 175 p.

LELAND, H. V., S. N. LUOMA, AND D. J. WILKES. 1977. Heavy metals and related trace elements. J. Water Pollut. Control Fed. 49: 1340–1369.

LENTSCH, J. W., T. J. KNEIP, M. E. WRENN, G. P. HOWELLS, AND M. EISENBUD. 1973. Stable manganese and manganese-54 distributions in the physical and biological components of the Hudson River estuary, p. 752–768. *In* D. J. Nelson [ed.] Radionuclides in ecosystems, Vol. 2, Proc. Third Nat. Symp. Radioecol., U.S. Atomic Energy Comm. Symp. Ser. CONF-710501.

LUCAS, H. F. JR., D. N. EDGINGTON, AND P. J. COLBY. 1970. Concentrations of trace elements in Great Lakes fishes. J. Fish. Res. Board Can. 27: 677–684.

MATHIS, B. J., AND T. F. CUMMINGS. 1973. Selected metals in sediments, water, and biota in the Illinois River. J. Water Pollut. Control Fed. 45: 1573–1583.

MATHIS, B. J., AND N. R. KEVERN. 1975. Distribution of mercury, cadmium, lead and thallium in a eutrophic lake. Hydrobiologia 46: 207–222.

MERLINI, M., F. ARGENTESI, A. BERG, A. BRAZZELLI, B. OREGIONI, AND G. POZZI. 1973. The biological pathway of zinc (^{65}Zn) in freshwater fish and its alteration by heavy metals, p. 285–306. *In* D. J. Nelson [ed.] Radionuclides in ecosystems, Vol. 1, Proc. Third Nat. Symp. Radioecol., U.S. Atomic Energy Comm. Symp. Ser. CONF-710501.

MERLINI, M., AND G. POZZI. 1977a. Lead and freshwater fishes: Part 1 — Lead accumulation and water pH. Environ. Pollut. 12: 167–172.

1977b. Lead and freshwater fishes: Part 2 — Ionic lead accumulation. Environ. Pollut. 13: 119–126.

MILANOVICH, F. P., D. W. WILSON, AND Y. YEH. 1975. Detoxifying effect of yellow substance on *E. coli* in media containing copper. Nature 253: 460–461.

MURPHY, B. R., G. J. ATCHISON, A. W. MCINTOSH, AND D. J. KOLAR. 1978. Cadmium and zinc content of fish from an industrially contaminated lake. J. Fish Biol. 13: 327–335.

PAGENKOPF, G. K., R. C. RUSSO, AND R. V. THURSTON. 1974. Effect of complexation on toxicity of copper to fishes. J. Fish. Res. Board Can. 31: 462–465.

PATTERSON, C. C., AND D. M. SETTLE. 1976. The reduction of orders of magnitude errors in lead analyses of biological materials and natural waters by evaluating and controlling the extent and sources of industrial lead contamination introduced during sample collecting, handling and analysis, p. 321–351. *In* Accuracy in trace analysis: sampling, sample handling, and analysis. U.S. Nat. Bureau Standards Spec. Publ. 422.

1977. Comparative distributions of alkalies, alkaline earths and lead among major tissues of the tuna *Thunnus alalunga*. Mar. Biol. 39: 289–295.

PENTREATH, R. J. 1976. Some further studies on the accumulation and retention of ^{65}Zn and ^{54}Mn by the plaice, *Pleuronectes platessa* L. J. Exp. Mar. Biol. Ecol. 21: 179–189.

PHILLIPS, D. J. H. 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments — a review. Environ. Pollut. 13: 281–317.

SCHROEDER, H. A., J. J. BALASSA, AND I. H. TIPTON. 1966. Essential trace metals in man: Manganese. J. Chron. Dis. 19: 545–571.

SKIDMORE, J. F. 1964. Toxicity of zinc compounds to aquatic animals, with special reference to fish. Quart. Rev. Biol. 39: 227–248.

STEVENS, J. D., AND B. E. BROWN. 1974. Occurrence of heavy metals in the blue shark *Prionace glauca* and selected pelagic fish in the N. E. Atlantic Ocean. Mar. Biol. 26: 287–293.

TING, R. Y. 1973. Distribution of Zn, Fe, Mn, and Sr in marine fishes of different feeding habits, p. 709–720. *In* D. J. Nelson [ed.] Radionuclides in ecosystems, Vol. 2, Proc. Third Nat. Symp. Radioecol., U.S. Atomic Energy Comm. Symp. Ser. CONF-710501.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1975. Scientific and technical assessment report on manganese. EPA-600/6-75-002. U.S. Government Printing Office, Washington, D.C. 71 p.

VANDERPLOEG, H. A., D. C. PARZYCK, W. H. WILCOX, J. R. KERCHER, AND S. V. KAYE. 1975. Bioaccumulation factors for radionuclides in freshwater biota. ORNL-5002. (Oak Ridge Nat. Lab., Environ. Sci. Div., Oak Ridge, TN, USA) 222 p.

ZITKO, P., W. V. CARSON, AND W. G. CARSON. 1973. Prediction of incipient lethal levels of copper to juvenile Atlantic salmon in the presence of humic acid by cupric electrode. Bull. Environ. Contam. Toxicol. 10: 265–271.